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Title: ***USE OF COX-2 INHIBITOR TO PREVENT T-CELL ANERGY INDUCED BY DENDRITIC CELLS WHEN IN CONTACT WITH COX-2 EXPRESSING CANCER CELLS***

Atty. Dkt.	305912	81476
	M#	**Client Ref**

including: Date: October 6, 2003

1. Specification: 14 pages 1A. ☒Claim: 1 pages 1B ☒ 1 Abstract pages
2. ☐ Specification in non-English language 3. ☒ Drawings: 6 sheet(s)

4. The invention ☐ was ☒ was not made by, or under a contract with, an agency of the U.S. Government.
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7. ☐ Attached:

8. This application is made by the following named inventor(s) **(Double check instructions for accuracy.):**

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9. NOTE: FOR ADDITIONAL INVENTORS, check box ☐ and attach sheet (PAT102A) with same information regarding additional invent rs.

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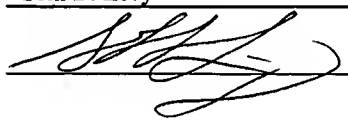
Sig: 

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John S. YU and Gentao LIU

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Title: ***USE OF COX-2 INHIBITOR TO PREVENT T-CELL ANERGY INDUCED BY DENDRITIC CELLS WHEN IN CONTACT WITH COX-2 EXPRESSING CANCER CELLS***

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APPLICATION FOR A
PROVISIONAL UNITED STATES PATENT
IN THE NAME OF

JOHN S. YU AND GENTAO LIU

for

USE OF COX-2 INHIBITOR TO PREVENT T-CELL ANERGY INDUCED BY DENDRITIC CELLS WHEN IN CONTACT WITH COX-2 EXPRESSING CANCER CELLS

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USE OF COX-2 INHIBITOR TO PREVENT T-CELL ANERGY INDUCED BY DENDRITIC CELLS WHEN IN CONTACT WITH COX-2 EXPRESSING CANCER CELLS

Introduction

Cancer cells can be recognized by the immune system and in some cases be eradicated, and the principal of the effective immune response is antigen-specific CD8+ cytotoxic T lymphocytes (CTLs) (1-3). This consensus is also supported by the evidence that several antigenic peptides complexed to class I major histocompatibility (MHC) molecules have been observed as tumor-associated antigens (TAA) (4-6). It has been shown that tumor-specific CTLs must recognize TAA on the same antigen-presenting cells (APCs) that cross-present the CD4+ helper T (Th) cell epitopes in a cognate manner, indicating the requirement for epitope linkage between Th and CTLs for induction of potent antitumor immune responses (7-9). Given the importance of Th responses in anti-tumor immunity, the ability of APCs to skew the tumor-specific Th cells towards Th-subset-1 (Th1) state of activation may play a significant role in augmenting the efficacy of any systemically mounted tumoricidal immune response.

Dendritic cells (DCs) are one of the most favorable candidates for induction of immunity against cancers. Because DCs are pivotal APCs equipped as follows; (i) capture and process TAA via dead cancer cells (7), (ii) display high levels of class I and II MHC-antigenic peptide complexes with co-expression of co-stimulatory molecules, such as CD54, CD80 and CD86 (10), (iii) produce high amount of interleukin (IL)-12 which polarizes Th cells toward Th1 (11-13). In addition, it has been shown that DCs express TNF family ligands such as TNF, Lymphotoxin-$\alpha_1\beta_2$, Fas ligand, and TNF-related apoptosis inducing ligand (TRAIL), and are fully equipped for an efficient direct apoptotic killing of cancer cells (14). Importantly, natural or therapy-induced high frequencies of tumor-infiltrating DCs have been associated with better clinical prognosis and/or regression of disease (15-17). Although these evidences are showing that DCs may play a critical role for prevention of cancers expansion in their rudiments as a member of the immune surveillance, the mechanism of cancers generating escape from the surveillance is not completely elucidated. DCs activate antigen-specific CTLs but also

maintain CTLs tolerance (18, 19). It has been explained that one of the mechanisms of DCs-mediated CTLs tolerance is caused in immature DCs and the maturation arrest on DCs is caused in their uptake of apoptotic cells (18-20). On the other hand, it has been shown that CTLs tolerance is mediated by CD83+ mature DCs which are induced by ProstaglandinE$_2$ (PGE_2) stimulation, and the interface between the mature DCs and CD4+ Th cells possesses a critical checkpoint of it (21, 22).

PGE_2 is one of the glioma-associated soluble factors which contribute to the cellular immune suppression (23-25), but the exact mechanism is unclear. PGE_2 is synthesized from arachidonic acid (AA) , and the critical stage in the PGE_2 formation is oxygenation of free AA by cyclooxygenase (COX) enzymes which have been identified two isoforms, COX-1 and COX-2 (26). COX-1 is constitutively present in many cell types, whereas COX-2 is inducible enzyme by several stimuli, such as IL-1-β and TNF-α (27-30). COX-2 overexpressing human malignant tumors have been reported including colonrectal cancer (31), lung cancer (32), bladder cancer (33), and malignant glioma (34). In addition, it has also been shown that COX-2 overexpression in glioma cells is associated with clinically worse prognosis (35). In this experiment, we demonstrate a human in vitro model that COX-2 overexpressing glioma generates Th2 polarization. A surfeit of PGE_2 from COX-2 overexpressing glioma caused DCs to enhance IL-10 and to suppress IL-12, resulting the tumor-infiltrating DCs were affected to bias naïve CD4+Th cells development toward Th2 that the tumor-specificity was given. Here we propose a new mechanism of tumor-specific CTLs tolerization induced by DCs which infiltrated into COX-2 overexpressing gliomas, and these findings may be warning against DC-based anti-cancer immunotherapy.

Materials and Methods

Tumor Cells. U-87MG human glioma cell line was obtained from American Type Culture Collection (Rockville, MD). LN-18 cell line was kindly provided by Dr. Erwin Van Meier (Emory University, Atlanta, GA). A primary cultured human glioma (MG-377) was established from surgical specimen of a glioblastoma patient at neurosurgical institute, Cedars-Sinai Medical Center.

Media and Reagents. Tumor cells were maintained at 37°C and 5% CO_2 in DMEM supplemented with 10% heat-inactivated FBS, 2mM glutamate, 10mM HEPES, 100U/ml penicillin, 100μg/ml streptomycin. 100% DMSO dissolved NS-398 (selective COX-2 inhibitor; Cayman Chemical, Anna Arbor, MI) was used at concentrations of 10μM. Recombinant human TNF-α (BioSource International, Camarillo, CA) at concentration of 20ng/ml and TRAIL (PeproTech Inc., Rocky Hill, NJ) at concentration of 300ng/ml were used.

COX-2 cDNA Plasmid and Transfection. The COX-2 cDNA was isolated from the pSG5-COX-2 plasmid which contains a full-length COX-2 cDNA in the pSG expression vector (kindly provided by Dr. Richard Kulmacz, University of Texas Medical School, Houston, TX) by EcoRI and XbaI digestion. COX-2 expression plasmid, designated pTracer-COX-2 was constructed as follows: COX-2 cDNA was inserted into the site between EcoRI and XbaI on pTracer-CMV2 expression vector (Invitrogen, Carlsbad, CA) which is available to express GFP fused to the selectable marker Zeocin. Lipofectamine 2000 (Invitrogen) and Plus Reagent (Invitrogen) were used for transfection to LN-18 according to the manufacture's protocols. The transfected cells of pTracer-COX-2, and the empty plasmid, pTracer-CMV2, were selected by Zeocin (Invitrogen), and stable transfectants were established (LN-18-COX-2, LN-18-EP).

Detection of Apoptotic Death. Tumor cells were treated with TNF-α and TRAIL for 24hr. Apoptotic death was detected using Annexin V-FITC Apoptosis Detection Kit I (Phamingen, San Diego, CA). Cells were stained with Annexin V-FITC (Ann V) and propidium iodide (PI) according to the manufacture's protocol. Early stage of apoptosis was defined by Ann V^+/ PI^- staining as analyzed by a fluorescence multicolor flow cytometry (FACScan; Becton Dickinson Immunocytometry Systems, San Jose, CA).

Western Blot. Samples were were extracted with buffer containing 1% Triton X-100, 150mM NaCl, 50mM Tris (pH 7.5) and 1mM PMSF. The cell debris was removed by centrifugation at 14,000g for 20 min and the supernatant was subjected to SDS-PAGE and loaded on a 7.5% polyacrylamide gel (Bio-Rad, Hercules, CA). Electrophoretic transfer to nitrocellulose membranes (Amersham Biosciences,

Piscataway, NJ) was followed by immunoblotting with a mouse IgG1 anti-COX-2 antibody (Phamingen) or a mouse IgG1 anti-beta-tublin (Sigma). This was followed by hybridization with a peroxidase-linked anti-mouse IgG antibody (Amersham Bioscience). The signal was detected by chemiluminiscence using ECL detection system (Amersham Bioscience) and Hyperfilm ECL (Amersham Bioscience).

DCs and lymphocytes Preparations. Human peripheral blood mononuclear cells (PBMC) were separated from peripheral blood of a healthy donor and the aforementioned glioblastoma patient using Ficoll-Hypaque density centrifugation. PBMC were resuspended in X-VIVO 15 serum-free medium (Cambrex; Santa Rosa, CA) and allowed to adhere to 24-well culture plate at 37°C. The non-adherent cells were removed after 2hr. CD4+ and CD8+ cells were isolated from these non-adherent cells with MiniMACS cell separation unit (Milenyi Biotec, Auburn, CA) and microbeads conjugated mouse monoclonal anti-human CD4 and CD8 antibody (Milenyi Biotec). CD4+ and CD8+ cells were used for mixed lymphocyte reaction (MLR) describing later. The adherent cells were subsequently cultured in X-VIVO 15 serum-free medium. 20ng/ml GM-CSF (BioSource International) and 10ng/ml IL-4 (BioSource International) were added to the cultures on days 0, 2, and 4. After 7-day culture, the floating cells were transferred to fresh plates with fresh X-VIVO 15 medium as immature DCs (iDCs). Several iDCs were cultured with 300ng/ml TRAIL and/or 0.1 □M PGE_2 (Sigma, St Louis, MO) and/or 20ng/ml TNF-α for 16hr (TRAIL-DCs, PGE_2-DCs, TRAIL/PGE_2-DCs, TRAIL/ TNF-α -DCs, TRAIL/ TNF-α /PGE_2-DCs).

Co-culture with Glioma Cells and DCs. Glioma cells were plated to 6-well culture plate at density of $1x10^6$ cells/well/2ml with fresh X-VIVO 15 medium. Several glioma cells were pre-treated with NS-398 for 8hr. The NS-398 treated glioma cells were cultured with TNF-α, TRAIL, and NS-398. The other cells were cultured with TNF-α and TRAIL. After 24hr culture with these agents, iDCs were put into the wells at the density of $3x10^5$cells/well, and were co-cultured with glioma cells for 16hr. Several iDCs were cultured with TNF-α /TRAIL or TNF-α /TRAIL/NS398 without tumor cells. Human glioma cell lines (U-87MG and LN-18) and stable

transfectants (LN-18-COX-2 and LN-18-EP) were co-cultured with healthy donor's iDCs, and MG-377 were co-cultured with autologous iDCs.

Flow Cytometric Analysis. Monoclonal mouse anti-human antibodies of PE-conjugated CD83, CD86, HLA-DR (Phamingen), FITC-conjugated CD11c (BioSource International), and uncojugated CCR7 (R&D Systems, Minneapolis, MN) were used for cell surface analysis of DCs. PE-conjugated $F(ab')_2$ goat anti-mouse immunoglobulins (DAKO Cytomation, Carpinteria, CA) was used for secondary antibody. Mouse IgG1 and IgG2a (Phamingen) were used for isotype control. DCs were stained with anti-CD86, HLA-DR, CD83, and CCR7 antibodies before staining with anti-CD11c antibody, and CD11c+ DCs were isolated with MiniMACS cell separation unit and microbeads-conjugated anti-FITC antibody (Milenyi Biotec).

Tumor cells were dyed red with PKH-26 (Sigma) before co-culturing with iDCs. DCs were stained with anti-CD11c-FITC antibody after 16hr co-culture, and were isolated as mentioned above. Isolated CD11c+ DCs were analyzed by FACScan where double positive cells indicate uptake of the tumor cells by DCs.

ELISA. PGE_2 ELISA kit (Cyman Chemical, Ann Arbor, MI) was used according to the manufacture's protocol for measurement in the supernatants of glioma cell cultures. IL-10 and IL-12 p70 ELISA kits (Phamingen) were used according to the manufacture's protocols for measurement in the supernatants of cultures of tumor cells or isolated CD11c+ DCs. CD40-CD40L interaction is a main trigger of IL-12 production by DCs (36). Based on this evidence, cells were co-cultured with NIH-CD40L cells (kindly provided by Dr. Gang Zeng, National Cancer Institute, NIH, MD; the ratio of NIH-CD40L : DCs = 1 : 1) for 16hr, and the supernatants of co-cultures were used as the samples. The lower detection limit of each kit was 7.8pg/ml. All samples and standards were run in duplicate.

MLR and ELISPOT CD11c+ DCs were co-cultured with autologous CD4+ and CD8+ lymphocytes with fresh X-VIVO 15 medium for 7 days (the initial stimulation; the ratio of DCs:lymphocytes = 1:40~50) in 6-well culture plate. The lymphocytes were transferred to fresh plates with fresh X-VIVO 15 medium and were re-stimulated with $1x10^5$ glioma cells for 48hr. After re-stimulation, Only CD4+ cells

were isolated again as mentioned above, and were re-suspended in fresh X-VIVO 15 medium.

Dual human INF-γ/IL-10 Eli-spot PVDF-enzymatic kit (Cell Sciences, Norwood, MA) and PVDF-bottomed 96-well plate (Cellular Technology, Cleveland, OH) were used for ELISPOT assay of the isolated CD4+ T cells. CD4+ T cells ($1x10^5$cells/100μl/well) were plated to INF-γ- or IL-10-capture antibody coated wells and were cultured at 37°C and 5% CO_2 for 20hr. All samples were run in duplicate. Spot-forming was analyzed by Alpha Imager Spot-reading System (Alpha Innotech, San Leandro, CA).

Statistics. Student's T test was used to compare with each result.

Results

Human Glioma Cells Overexpress COX-2 Protein and Highly Produce PGE_2. Expression of COX-2 protein in human glioma cells was examined by Western blot (Fig. 1 A). COX-2 protein was detected in U-87MG, LN-18, and MG-377, and the treatment with TRAIL and TNF-α highly induced COX-2 expression in U-87MG and MG-377. COX-2 inhibitor did not extinguish the COX-2 expression. There was no major difference of COX-2 expression in each group of LN-18.

PGE_2 in the culture supernatants was measured by ELISA (Fig. 1 B). The treatment with TRAIL and TNF-α caused a significant increase in PGE_2 in the supernatants of COX-2 overexpressing cells U-87MG and MG-377 compared with each control ($p<0.01$), and addition of NS-398 into their cultures significantly reduced it ($p<0.01$). Although PGE_2 was detectable in the supernatant of LN-18, there was no significant difference in each group.

DCs Efficiently Phagocytose Apoptotic Glioma Cells. Apoptotic death was analyzed using Annexin V-FITC Apoptosis Detection Kit and FACScan where Ann V^+/ PI^- cells indicate early stage of apoptosis and Ann V^+/PI^+ cells also indicate dead cells. The treatment of glioma cells with TRAIL and TNF-α caused a significant increase in the ratio of apoptotic death ($p<0.01$) and Ann V^+/PI^+ death ($p<0.01$) compared with the control in each group (Fig 2 A). The treatment of iDCs with TRAIL and TNF-α did not

induce their death (data not shown). NS-398 treatment did not cause a change of the death ratio in each glioma (data not shown).

Glioma cells were dyed red with PKH-26 before co-culturing with iDCs. DCs were stained with anti-CD11c-FITC antibody after 16hr co-culture, and isolated CD11c+ DCs were analyzed by FACScan where double positive cells indicate DCs phagocytosed glioma cells. It has been shown that DCs can phagocytose apoptotic cells by the recognition of phosphatidylserine which can be detected by Annexin V (37, 38). In fact, a significant increase of Ann V^+ death in glioma caused a significant increase ($p<0.01$) in the ratio of DCs which phagocytosed glioma cells compared with each control (Fig 2 B).

DCs Can Be Matured by The Treatment with* TNF-α *after Phagocytosis of Glioma Cells. DCs were stained with anti-CD86, HLA-DR, CD83, CCR7, and CD11c antibody. CD11c+ DCs were isolated with cell separation unit and microbease-conjugated anti-FITC antibody. Single-cultured iDCs were highly expressing CD86 and HLA-DR, but did not express CD83 or CCR7 which are maturation markers of DCs (Fig 3). The co-stimulation of TRAIL and/or PGE_2 on single-cultured iDCs up-regulated CD86 expression (geometric mean; iDCs:647, TRAIL-DCs:1086, PGE_2-DCs:2779, TRAIL/PGE_2-DCs:1176), but they did not induce CD83 or CCR7 expression (Fig 3). The co-stimulation with TNF-α on single-cultured iDCs induced both CD83 and CCR7 expressions, and co-stimulation with TNF-α and PGE_2 strongly up-regulated these maturation markers (Fig 3). These data indicate that stimulation of TNF-α is required for maturation of immature DCs.

Expression of CD86 on DCs containing glioma cells was significantly down-regulated compared with single-cultured iDCs (geometric mean; co-cultured DCs: 317-480, single cultured DCs: 647-4720; $p< 0.01$), but there was no significant difference on the ratio of CD86 positive cells (co-cultured DCs: 95-98%, single cultured DCs: 96-98%) (Fig 3). Importantly, both CD83 and CCR7 expressions on co-cultured DCs were highly expressed as well as single-cultured TRAIL/ TNF-α /PGE_2-DCs (Fig 3). There was no statistical difference on geometric means of the expressing markers in the variation of co-cultured glioma cells or NS-398 treatment (Fig 3). These data indicate that DCs can be completely matured by the effect of TNF-α stimulation, even if they contained apoptotic glioma cells.

Inhibition of COX-2 Protein in Gliomas Is Effective in IL-10 Reduction and IL-12p70 Augmentation in Tumor-infiltrating DCs. IL-10 and IL-12p70 in the supernatants of cultures were measured by ELISA, and the results were statistically compared with NS-398(-) single-cultured DCs (Fig. 4). COX-2 inhibitor for single-cultured DCs did not effect to change IL-10 or IL-12p70 production. U-87MG ($p<0.01$), LN-18 ($p<0.05$) and MG-377 ($p<0.05$) affected the co-cultured DCs to enhance IL-10, and also U-87MG ($p<0.01$) and LN18 ($p<0.05$) affected to suppress IL-12p70. COX-2 inhibition in gliomas remedied the IL-10 surplusage and IL-12 deficiency in the co-cultured DCs. There was no detectable IL-10 or IL-12p70 in the supernatants of glioma cell cultures (data not shown). These data are indicating that COX-2 expression in glioma cells may relate with IL-10 augmentation and/or IL-12 reduction in co-cultured DCs.

COX-2 Inhibitor in Glioma Has a Capacity for DCs-mediating Th Polarization to Bias Toward Th1. MLR by co-culturing with CD4+ ,CD8+ lymphocytes and autologous DCs was performed, and Th cell polarization was confirmed by ELISPOT assay (Fig. 5A-F). The results were statistically compared with the lymphocyte which was initially-stimulated by NS-398(-) single-cultured DCs. The DCs co-cultured with glioma cells induced tumor-specific Th response which was polarized toward Th2, and inhibition of COX-2 in gliomas resulted in polarization toward Th1 (Fig. 5A-D).

The DCs co-cultured with autologous tumor (MG-377) did not change the tumor-specific Th response compared with their single-cultured DCs (Fig. 5E, F), indicating that glioma patients already had a similar Th response against their tumors which could be induced by the tumor-infiltrating DCs. However, this Th response was polarized toward Th2. The DCs co-cultured with COX-2-inhibited glioma cells changed Th polarization toward Th1 (Fig. 5E, F). These data indicate that COX-2 inhibitor in glioma has a capacity for DCs-mediating Th polarization to bias toward Th1.

Discussion

Natural or therapy-induced high frequencies of tumor-infiltrating DCs have been associated with better clinical prognosis and/or regression of disease (15-17). DCs inoculated in the brain, considered an immunologically privileged site due to the lack of lymphatic drainage and the nature of the blood brain barrier (BBB) (39),

migrated to the lymph nodes and induced anti-tumor immunity against brain tumors due to the stimulation of CTLs activity (16, 17). DCs migration from periphery to lymphoid organs is associated with expression of chemokine receptor 7 (CCR7) which is one of the maturation markers of DCs (40), indicating several tumor-infiltrating DCs do not fall into a maturation arrest after the DCs phagocytose apoptotic tumor cells. In our experiment, TNF-α stimulation for tumor-infiltrating DCs could induce CD83+/CCR7+ mature DCs after they phagocytosed apoptotic cells, suspecting the DCs can migrate to lymph nodes and can present the TAA to T cells. DCs containing tumor cells down-regulate the expression of co-stimulatory molecules such as CD86. The down-regulation of co-stimulatory molecules on DCs may be associated with the limited efficacy of anti-tumor immunity, but it is difficult to explain the mechanism of tumor generating escape from the DCs-mediating immune surveillance only in this reason.

It has been reported that tumor culture supernatant-exposed DCs lacked the capacity to produce IL-12 and did not acquire full allostimulatory activity (41, 42). Based on these evidences, the authors had a hypothesis that one of the suppressive factors in tumor culture supernatant for DCs may be PGE_2 because of the evidences that PGE_2 induced mature DCs were impaired IL-12 production and were associated with antigen-specific CTLs tolerance (21, 22). It has been known that PGE_2 synthesized by COX enzymes such as COX-1 and COX-2 (26) is one of the glioma-associated soluble factors which contribute to the cellular immune suppression (23-25). However, the mechanism of the cellular immune suppression that glioma-associated PGE_2 induces has not been completely elucidated. Ironically, TNF-α which induces DCs maturation, and TRAIL which induces tumor cell apoptosis, both of which are equipped by DCs, up-regulated COX-2 expression in glioma cells. Therefore, TNF-α and TRAIL induced PGE_2 overproduction from COX-2 overexpressing gliomas. Infiltration of DCs into TNF-α /TRAIL treated glioma caused DCs to enhance IL-10 and to suppress IL-12, resulting the DCs were affected to bias naïve CD4+Th cells development toward Th2 in both of allogenic and autologous models. This tendency was strongest on the highest PGE_2 producing glioma U-87MG. Interestingly, the autologous model revealed that glioma patients already had a Th2

response against their tumors similar to what could be induced by the DCs co-cultured with autologous tumor. The DCs infiltrating into COX-2 inhibited glioma polarized the Th cells toward Th1. COX-2 overexpression in glioma cells was associated with clinically worse prognosis (35), and the supplement of recombinant IL-12 was required for induction of potent therapeutic efficacy on DCs-based glioma immunotherapy (43). These evidences are completely compatible with our results, because the ability of tumors to skew the tumor-specific Th cells towards Th2 polarization should play a significant role in limiting the efficacy of CTLs-based tumoricidal immune response and IL-12 should play a critical role for Th polarization toward Th1 in DCs-mediating immunity of tumor-bearing hosts.

The relationship between PGE_2 in glioma products and the Th cell polarization mediated by the tumor-infiltrating DCs was confirmed using pTracer-COX-2 stable transfectant, LN-18-COX-2 and pTracer-CMV2 stable transfectant, LN-18-EP where LN-18-EP expects to produce the same immune suppressants except PGE_2 compared with LN-18-COX-2. In this experiment, LN-18-COX-2 highly produced PGE_2, and completely affected DCs to bias Th cells development toward Th2. Addition of soluble PGE_2 into culture supernatant of LN-18-EP affected Th2 polarization same as LN-18-COX-2. Furthermore, COX-2 inhibitor could suppress PGE_2 that LN-18-COX-2 produces, and the DCs infiltrating them could polarize the Th cells toward Th1, indicating the PGE_2 in glioma product is a major target of glioma generating tumor-specific CTLs tolerance mediated by glioma-infiltrating DCs. These results are showing one of the mechanisms of escape from the immune surveillance what glioma plays in their rudiments. The elucidation of mechanism on immunological tolerance against progressive cancers is required for induction of effective anti-cancer immunity, and one of the essential mechanisms is COX-2 overexpression in cancers.

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While the description above refers to particular embodiments of the present invention, it will be understood that many modifications may be made without departing from the spirit thereof. The accompanying claims are intended to cover such modifications as would fall within the true scope and spirit of the present invention. The presently disclosed embodiments are therefore to be considered in all respects as illustrative and not restrictive, the scope of the invention being indicated by the appended claims, rather than the foregoing description, and all changes that come within the meaning and range of equivalency of the claims are therefore intended to be embraced therein.

WHAT IS CLAIMED IS:

1. A method for enhancing the effect of a dendritic cell therapy, comprising:

administering a dendritic cell therapy; and

administering a COX-2 inhibitor in conjunction with said dendritic cell therapy to enhance an anti-tumor response.

ABSTRACT

ProstaglandinE$_2$ (PGE_2) induces CD83+ mature dendritic cells (DCs) which maintain antigen-specific cytotoxic T lymphocytes (CTLs) tolerance as well as immature DCs. The interface between mature DCs and CD4+ helper T (Th) cells has been shown to possess a critical checkpoint of it. PGE_2 synthesized by cyclooxygenes (COX) enzymes such as COX-1 and COX-2 is one of the glioma-associated soluble factors which contribute to the cellular immune suppression, but the exact mechanism is unclear. In this experiment, human glioma cell lines U-87MG, LN-18 and primary cultured glioma (MG-377) were co-cultured with DCs as a human in vitro model of tumor-infiltrating DCs. DCs could efficiently phagocytose apoptotic glioma cells, and could be matured by TNF-α stimulation. Infiltration of DCs into glioma caused DCs to enhance IL-10 and to suppress IL-12, resulting the DCs were affected to polarize Th cells toward Th-subset-2 (Th2). COX-2 inhibitor could suppress PGE_2 that COX-2 overexpressing glioma produces, and the DCs infiltrated into COX-2 suppressed glioma could polarize Th cells toward Th-subset-1 (Th1), indicating PGE_2 in glioma products is a major target of glioma generating CTLs tolerance. These results indicate a new mechanism of tumor-specific CTLs tolerization induced by DCs infiltrated into COX-2 overexpressing gliomas.

Fig 1 A

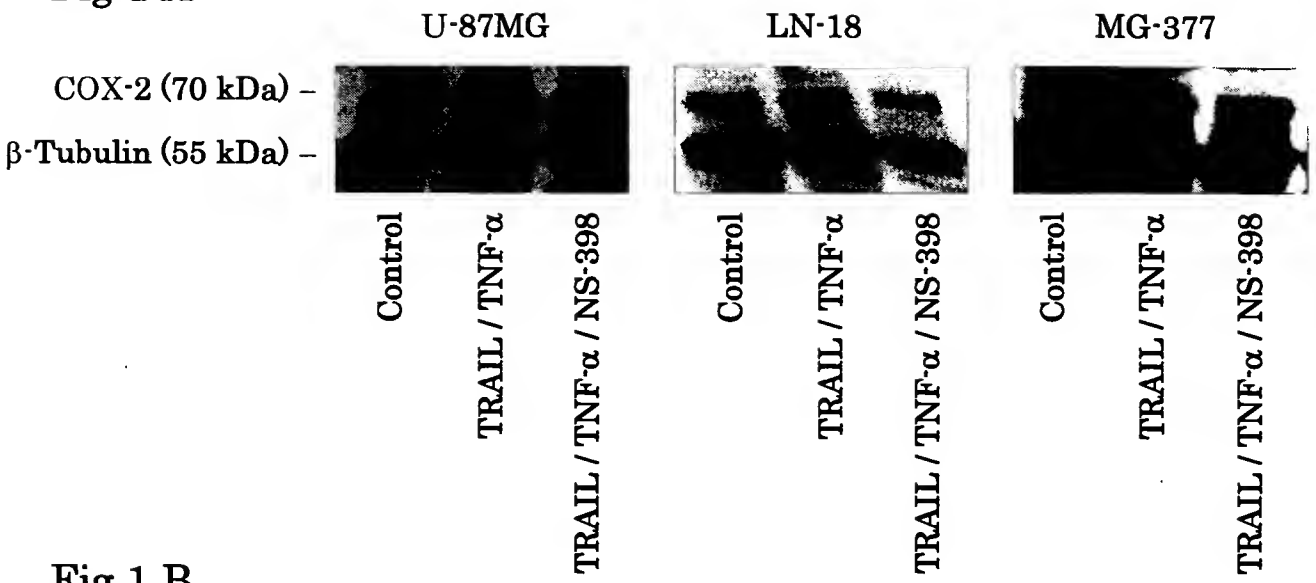

U-87MG
LN-18
MG-377
COX-2 (70 kDa) –
β-Tubulin (55 kDa) –
Control
TRAIL / TNF-α
TRAIL / TNF-α / NS-398
Control
TRAIL / TNF-α
TRAIL / TNF-α / NS-398
Control
TRAIL / TNF-α
TRAIL / TNF-α / NS-398

Fig 1 B

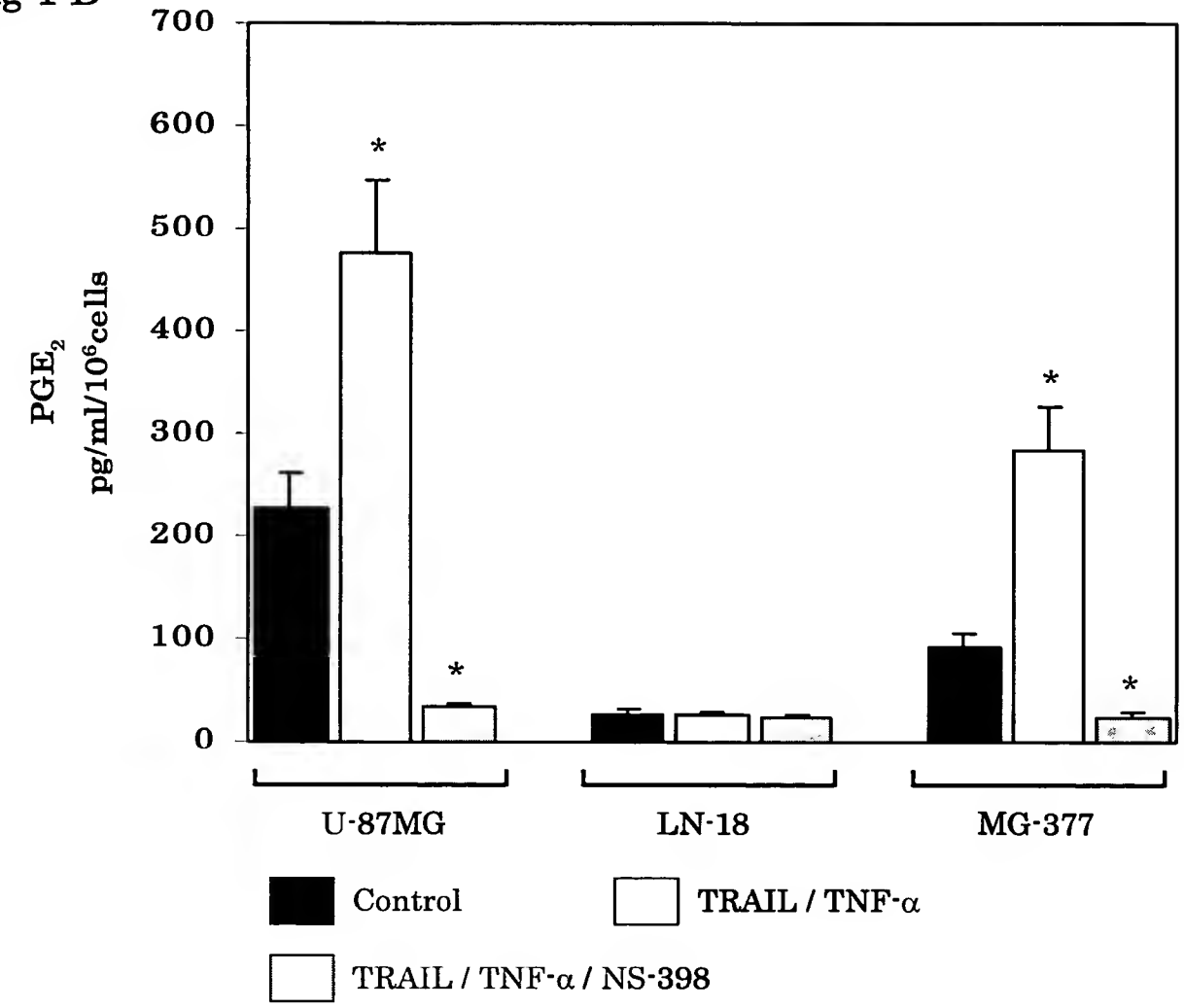
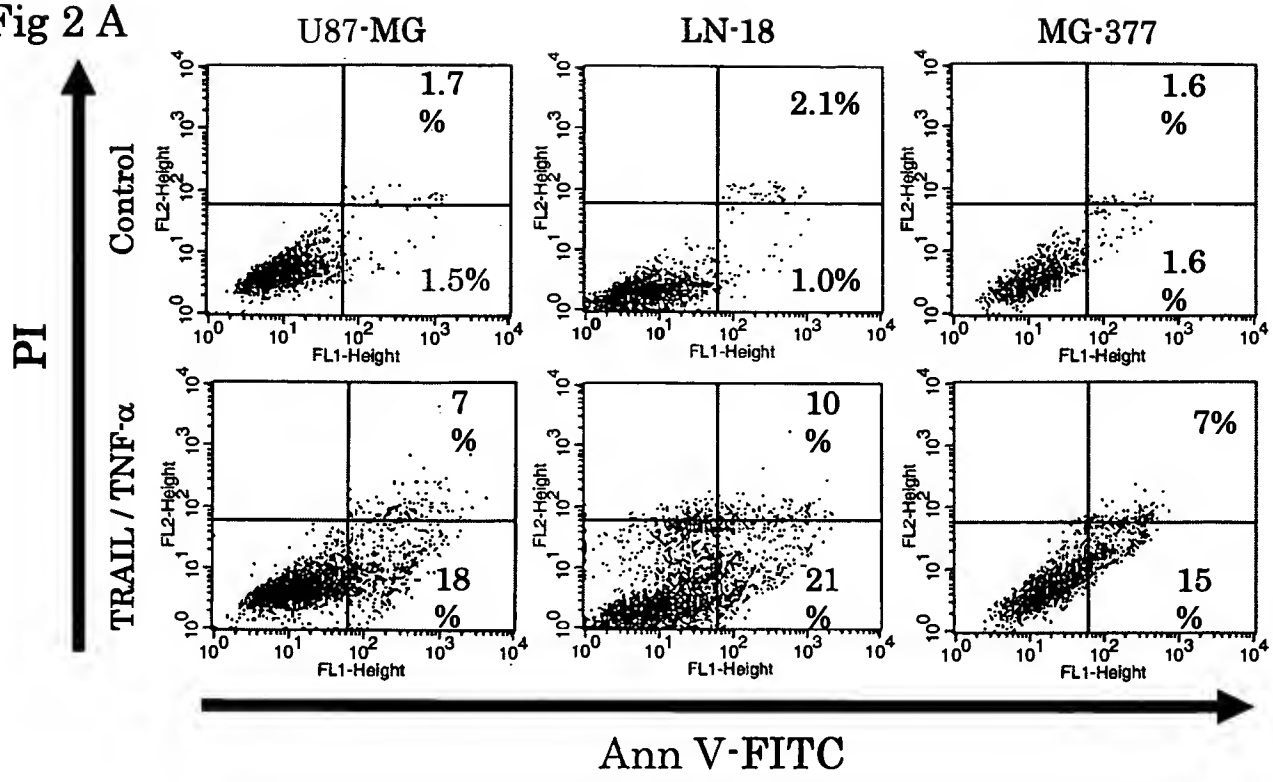

700
600
500
400
300
200
100
0
PGE2
pg/ml/10^6cells
*
*
*
*
U-87MG
LN-18
MG-377
Control
TRAIL / TNF-α
TRAIL / TNF-α / NS-398

Fig 2 A


U87-MG
LN-18
MG-377
PI
Control
TRAIL / TNF-α
1.7 %
1.5%
2.1%
1.0%
1.6 %
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7 %
18 %
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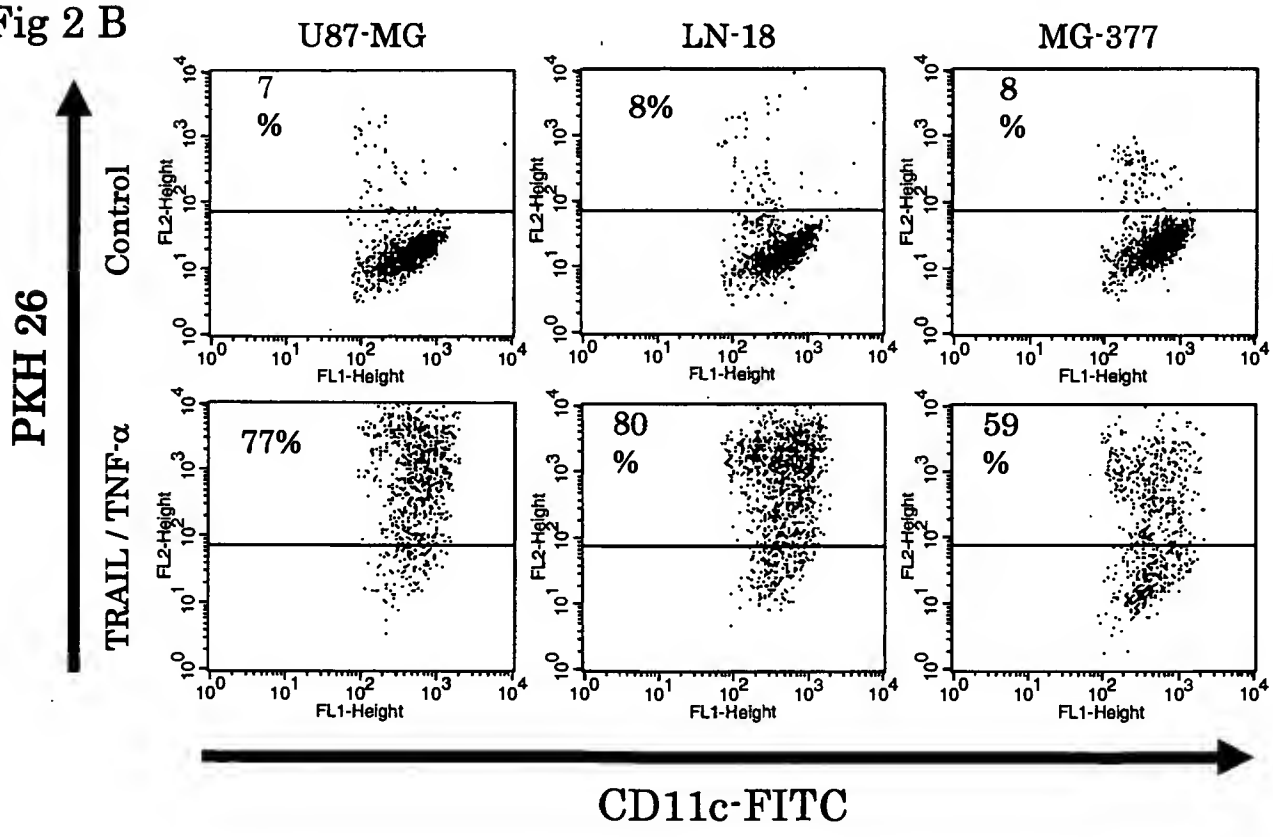
21 %
7%
15 %
FL2-Height
FL1-Height
Ann V-FITC

Fig 2 B


U87-MG
LN-18
MG-377
PKH 26
Control
TRAIL / TNF-α
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8%
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FL2-Height
FL1-Height
CD11c-FITC

Fig 3

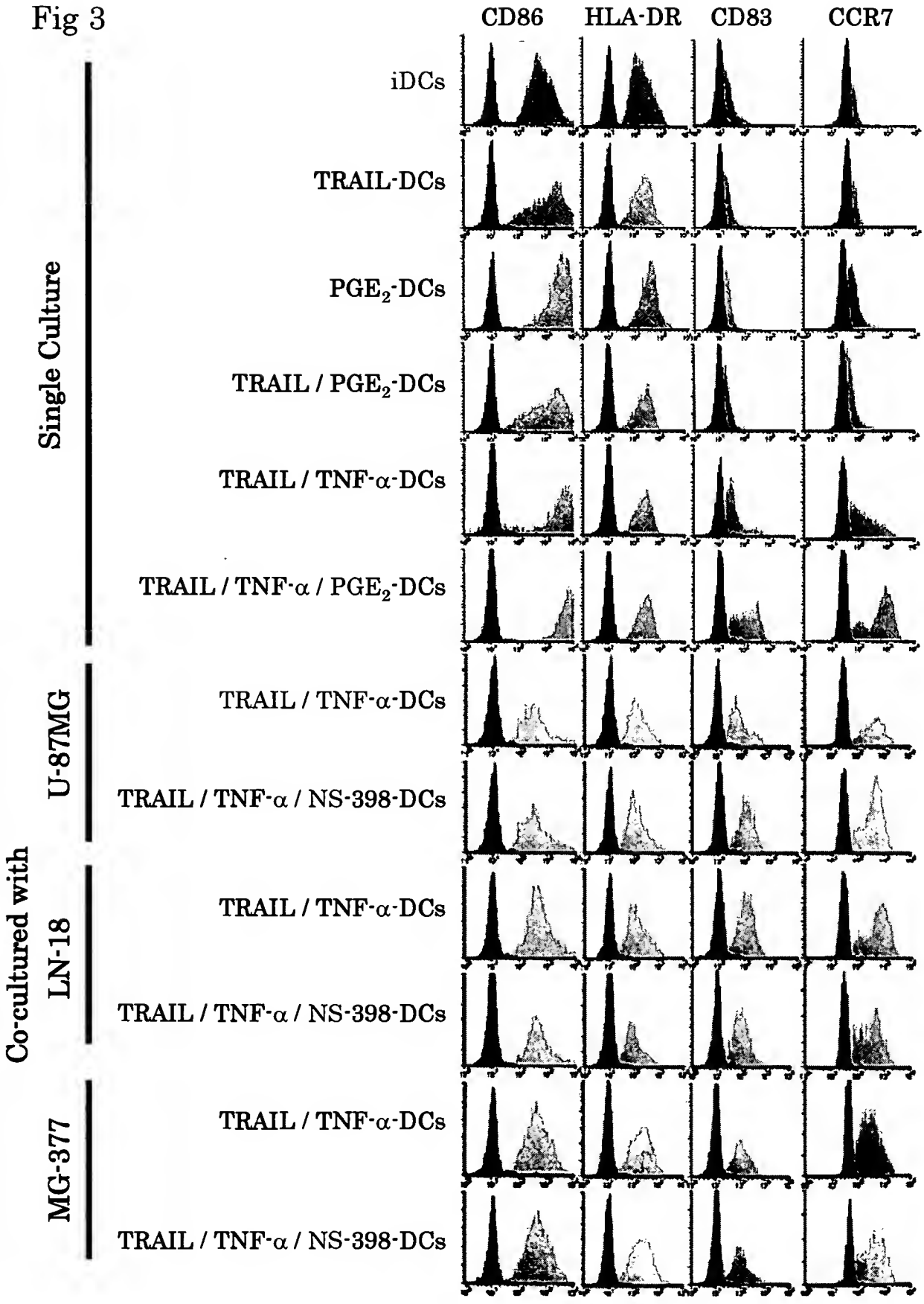

CD86
HLA-DR
CD83
CCR7
Single Culture
iDCs
TRAIL-DCs
PGE2-DCs
TRAIL / PGE2-DCs
TRAIL / TNF-α-DCs
TRAIL / TNF-α / PGE2-DCs
Co-cultured with
U-87MG
TRAIL / TNF-α-DCs
TRAIL / TNF-α / NS-398-DCs
LN-18
TRAIL / TNF-α-DCs
TRAIL / TNF-α / NS-398-DCs
MG-377
TRAIL / TNF-α-DCs
TRAIL / TNF-α / NS-398-DCs

Fig 4

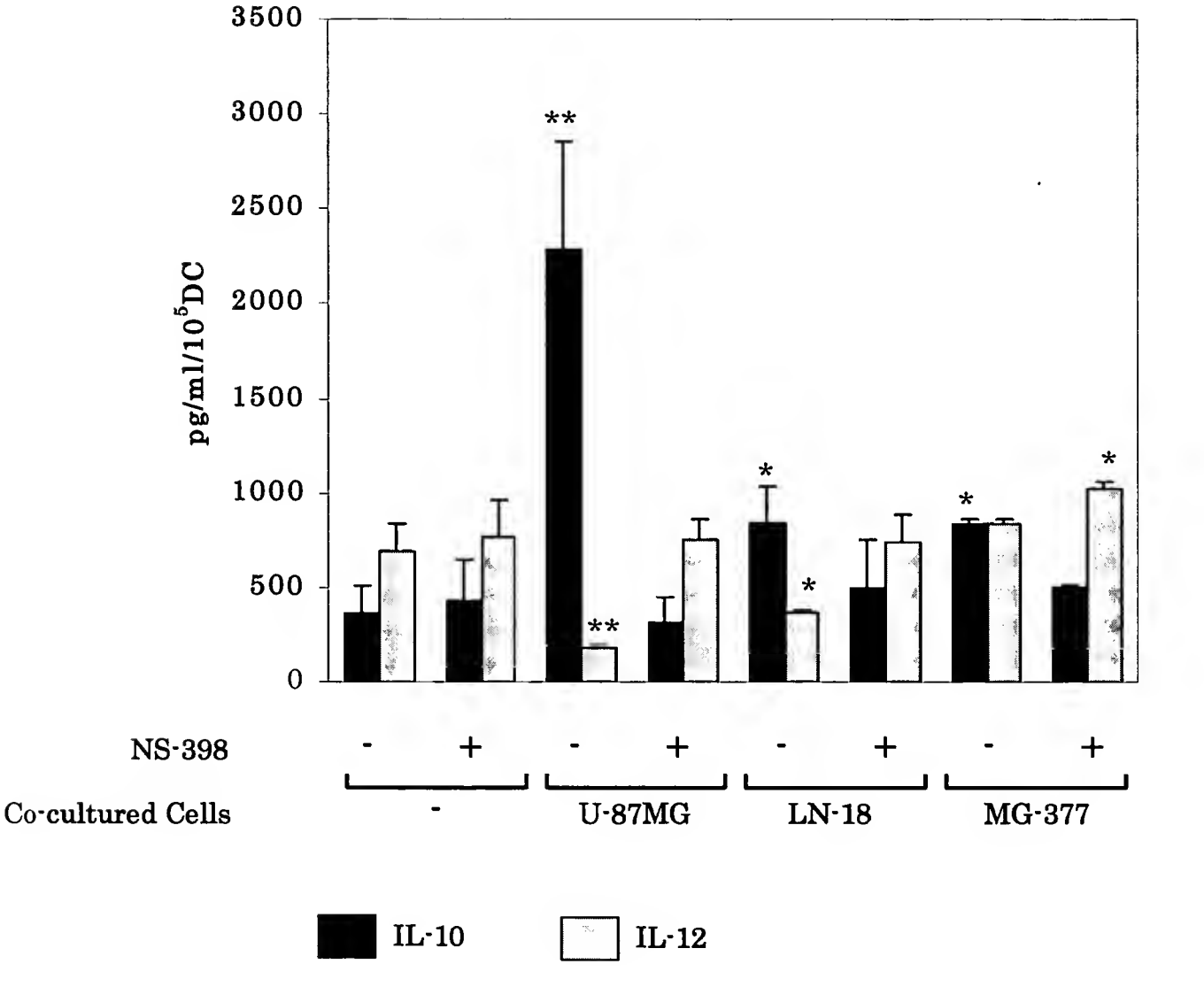

3500
3000
2500
2000
1500
1000
500
0
pg/ml/10^5DC
**
**
*
*
*
*
NS-398
- + - + - + - +
Co-cultured Cells
- U-87MG LN-18 MG-377
IL-10
IL-12

Fig 5 A

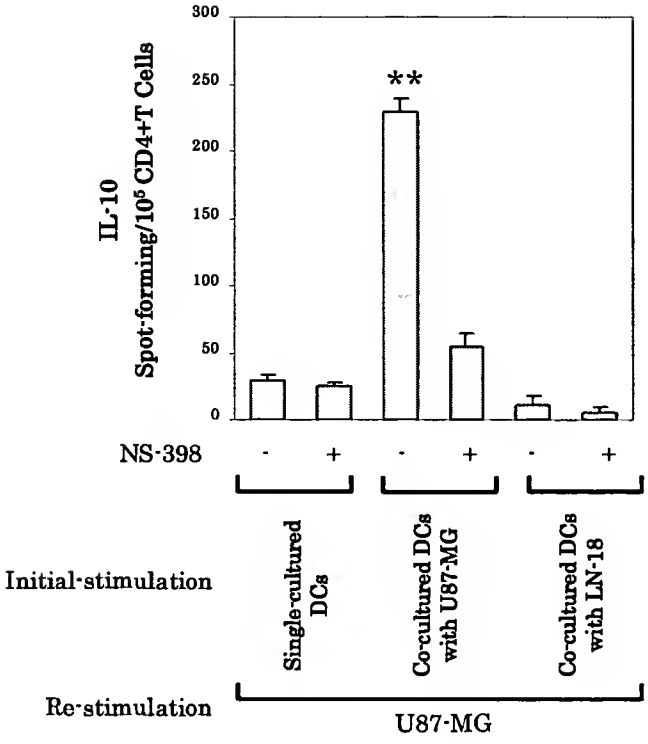


Fig 5 B

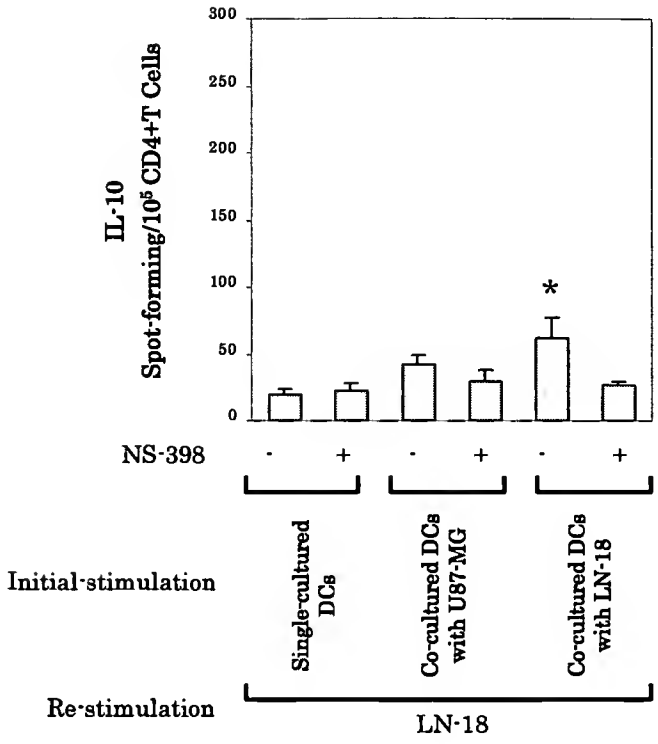


Fig 5 C

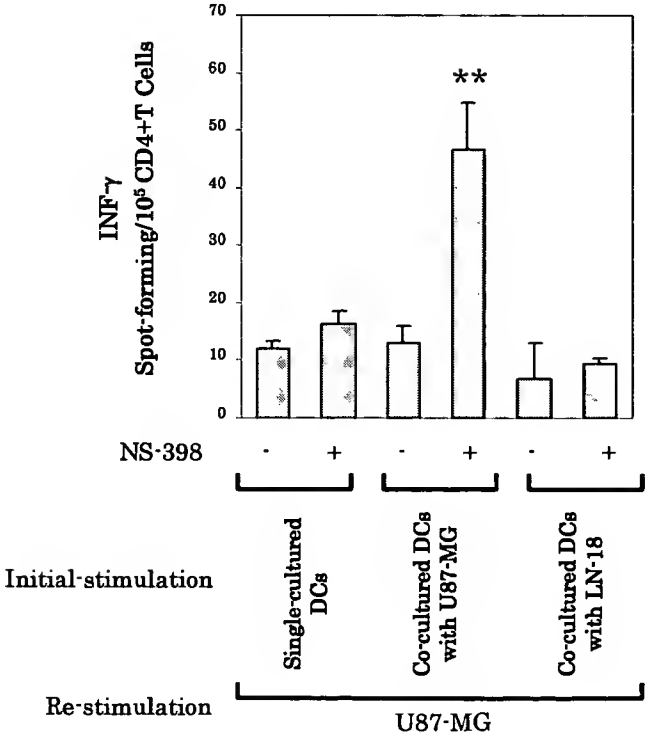


Fig 5 D

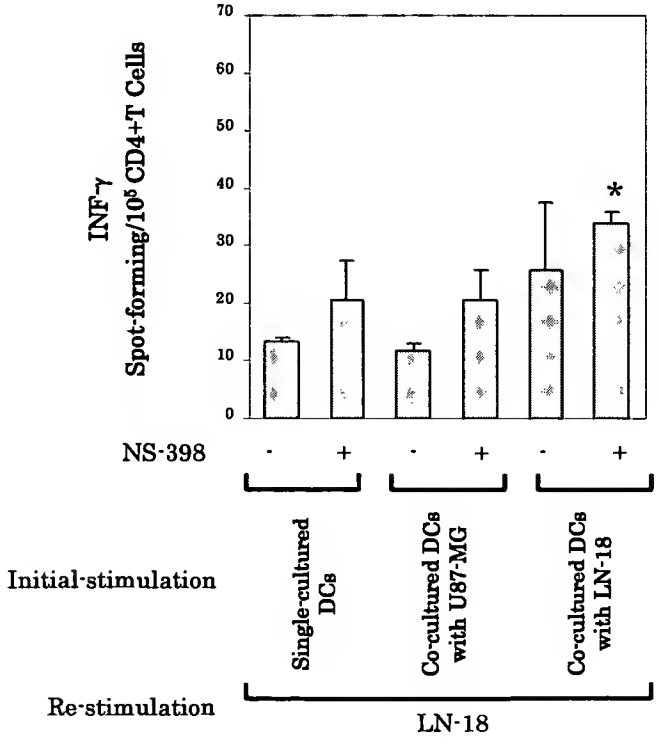


Fig 5 E

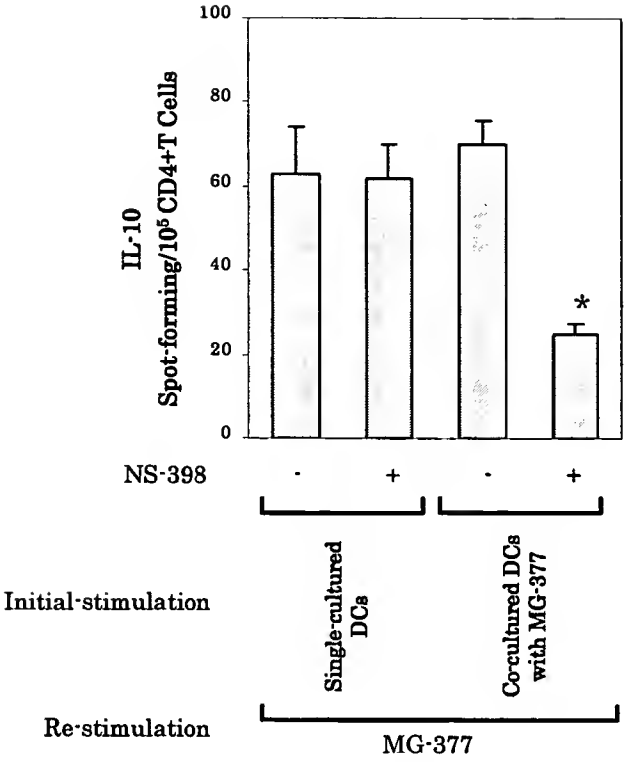
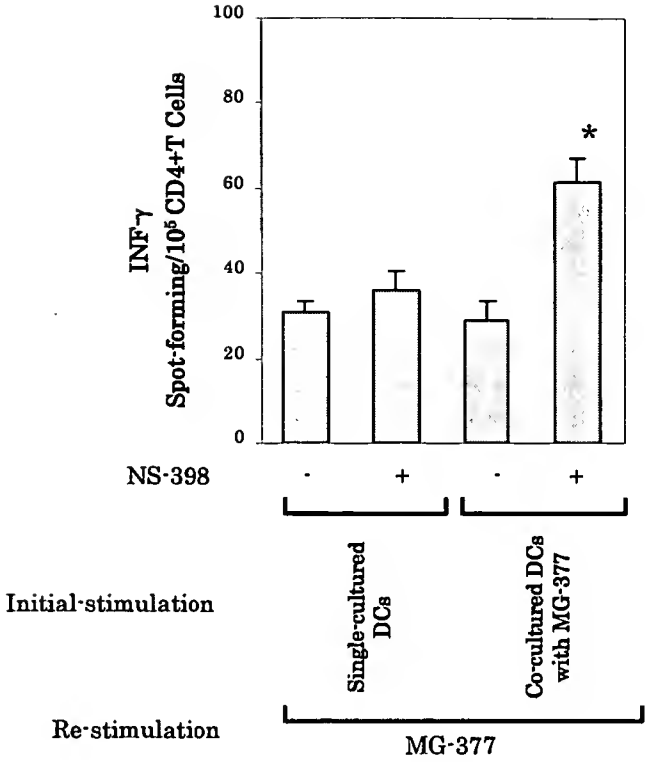

IL-10
Spot-forming/10⁵ CD4+T Cells
100
80
60
40
20
0
*
NS-398
-
+
-
+
Initial-stimulation
Single-cultured DCs
Co-cultured DCs with MG-377
Re-stimulation
MG-377

Fig 5 F


INF-γ
Spot-forming/10⁵ CD4+T Cells
100
80
60
40
20
0
*
NS-398
-
+
-
+
Initial-stimulation
Single-cultured DCs
Co-cultured DCs with MG-377
Re-stimulation
MG-377